Hepatoprotective effect of Chloroform extract of *Argemone mexicana* against Carbon tetrachloride-induced hepatotoxicity in rats

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Abstract

**Aim:** To investigate the hepatoprotective activity of chloroform extract of *Argemone mexicana* against carbon tetrachloride (CCl₄)-induced hepatic injury using *in-vivo* animal model.

**Materials and methods:** Chloroform extract of *Argemone mexicana* (100 and 200 mg/kg body weight) was administered daily in experimental animals. The hepatoprotective efficacy of Chloroform extract of *Argemone mexicana* (100 and 200 mg/kg) was investigated against CCl₄ -induced hepatotoxicity, elevated liver enzymes [ALT (alanine aminotransferase), AST (aspartate aminotransferase), and alkaline phosphatase (ALP)], and total protein (TP) contents in the serum. Moreover, Chloroform extract of *Argemone mexicana* -aided antioxidant defense against hepatotoxic insult of CCl₄ was measured by evaluating a number of anti-oxidative biomarkers including reduced malondialdehyde (MDA) in the serum.

**Results:** Results showed that the exposure of experimental animals to CCl₄ did induce significant hepatotoxicity compared to the non-induced (untreated) group. The oral administration of Chloroform extract of *Argemone mexicana* demonstrated a significant dose-dependent alleviation in the liver enzymes (AST, ALT, and ALP) and reduced MDA levels in the serum of treated animals compared to the animals without treatment. The resulting data showed that the administration of Chloroform extract of *Argemone mexicana* decreased the serum levels of ALT, AST, and ALP compared to the CCl₄-induced group.

**Conclusions:** The results of this study indicate that the Chloroform extract of *Argemone mexicana* may prevent CCl₄-induced liver injury.

**Keywords:** Chloroform extract of *Argemone mexicana*; antioxidant; antihepatotoxicity
Introduction

The liver plays an important role in the metabolism and elimination of various exogenous and endogenous compounds. It is involved with almost all the biochemical pathways to growth, fight against the disease, nutrient supply, energy provision, and reproduction. Liver cancer is the most typical fifth type of cancer in men and seventh most prevalent type in women that is caused by viral and some exogenous environmental toxins. Liver is the major organ of our body that is involved in detoxification and metabolism to maintain homeostasis in body against external noxious challenges. This detoxification process is very important because otherwise the exogenous chemicals might cause over production of free radicals that are harmful to liver normal functions. The ingestion of CCl4 in rat modal causes necrosis that further lead to steatosis, fibrosis, and cirrhosis and finally transformed into the hepatocellular carcinoma.

There is vast evidence indicating that herbal extracts from edible and medicinal plants exhibit strong antioxidant activity that could act against hepatic toxicity caused by various toxicants.

Now a day, the use of herbal natural products has enhanced worldwide attention. Many herbal products are claimed to assist in a healthy lifestyle. Medicinally, herbal drugs have made a major contribution to the treatment of hepatotoxic activity. Traditionally rhizome of Nardostachys jatamansi is used as antidiabetic activity, anti-cancer activity, anti-hiv activity, cns related activities, wound recovering action, anti microbial activity, antioxidant activity, anti-inflammatory, analgesic, antipyretic activity, hepatoprotective activity, anti-fertility activity, antiallergic activity, nematicidal activity, allelopathic effect, antihelmintic activity, larvicidal activity, antifeedant action. Therefore the present study was designed to evaluate the hepatoprotective activity of Chloroform extract of Argemone mexicana against carbon tetrachloride (CCl4)-induced hepatic injury using in-vivo animal model.

Materials and Methods

Collection of plant materials

Whole plant materials of Argemone mexicana L. were collected and plant materials were washed with distilled water to remove the adhering unwanted particles. They were shade dried for 2-3 days in sun but under the shade, and finally pulverized in to coarse powder. It was stored in a well closed container free from environmental climatic changes till usage.

Extraction using maceration process

The shade dried whole plant materials of Argemone mexicana L. were extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place. Plant material were extracted in four solvents of different polarity viz water, methanol, ethyl acetate and chloroform. Powdered plant material (100 gm)
was extracted by maceration method. Each time before extracting with next solvent, the powdered material was air dried and then subjected to further extraction. After the effective extraction, which they were filtered using Whatman filter paper No.1. Then the extracts was evaporated to solventlessness to yields crude extracts to produce a sticky material, and further transferred into sterile bottles and refrigerated until use.

**Phytochemical screening**

Different extracts of *Argemone mexicana* was subjected to phytochemical screening for the detection of various phyto-constituents.

**Animals**

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. The study protocols were approved by the Institutional Animal Ethics Committee (IAEC).

**Drugs and Chemicals**

CCl₄ and Silymarin (Sigma chemicals, USA) were used in present study. All other chemicals and other biochemical used in the experiments were of analytical grade from different firms.

**Experimental designs**

**Experimental design and treatment protocol**

Rats were acclimated to animal laboratory conditions at 25°C, 55% humidity, and a 12 h:12 h light-dark cycle for seven days prior to testing. Water was supplied ad libitum, and the rats were fed a basal diet for the entirety of the study.

**CCl₄-induced hepatotoxicity**

**Group –I:** Normal control (0.5% CMC 1 ml/kg, p.o.)

**Group –II:** Rats were subcutaneously injected with CCl₄ (2 mL/kg b.wt.)

**Group -III:** Rats were subcutaneously injected with CCl₄ (2 mL/kg b.wt.) and silymarin 10 mg/kg. Silymarin is the most used natural constituent for the healing of hepatic diseases worldwide due to its antifibrotic, anti-inflammatory, and antioxidant activities. Silymarin functions by stabilizing biological membranes and increasing protein synthesis Therefore, it is used as a standard drug around the world for hepatoprotective experiments.

**Group –IV:** Rats were subcutaneously injected with CCl₄ (2 mL/kg b.wt.) and chloroform extract of *Argemone mexicana* 100mg/kg

**Group –V:** Rats were subcutaneously injected with CCl₄ (2 mL/kg b.wt.) and chloroform extract of *Argemone mexicana* 200mg/kg

At the end of four weeks, food (but not water) was withheld from all animals for 12 h. All rats were sacrificed with isoflurane. Blood samples were collected in clean centrifuge tubes via cardiac puncture. The samples were centrifuged at 3000 rpm for 15 min to separate
the serum. The serum was carefully removed and transferred into lavender test tubes and solidified at 20°C until utilization for biochemical experiments.

**Biochemical Evaluation in Serum**

Serum aspartate aminotransferase (AST), Serum alanine transaminase (ALT), Alkaline Phosphate (ALP) was determined by using standard kits from Merck, Mumbai, India. All estimation was carried out using UV spectrophotometer (Shimadzu, India).

**Determination of antioxidant defense**

The antioxidant activity of Chloroform extract of *Argemone mexicana* (100 and 200 mg/kg) was evaluated by investigating the levels of malondialdehyde (MDA) in the serum specimens of all experimental groups. The level of lipid peroxidation was determine by the measuring the level of MDA using a previously established method14.

**Statistical analysis**

Variables of interest were entered and all data analyzed using GraphPad Instant 3.06 software version 14 for windows XP (Microsoft Corporation). All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable p<0.05 was considered statistically significant, compared with vehicle followed by Dunnett’s test.

**Results**

**Qualitative Phytochemical Tests**

The results from qualitative tests gave information on different classes of phytochemicals that alkaloids, flavonoids, diterpenes, phenol and saponins are present in the *Argemone Mexicana*.

**Effect of Chloroform extract of Argemone mexicana (100 and 200 mg/kg) on CCl4-induced hepatotoxicity and liver enzymes**

The hepatoprotective efficacy of Chloroform extract of *Argemone mexicana* (100 and 200 mg/kg) was determined by evaluating the levels of ALT, AST, ALP, and TP in the serum samples of all experimental animals. Results indicated that ingestion of CCl4 in Wistar albino rats caused a significant (P < 0.05) increase in the levels of ALT and AST in the serum specimen compared to the untreated group (Table 1). Results also indicated that treatment of CCl4-intoxicated rats with Chloroform extract of *Argemone mexicana* resulted in a significant (P < 0.05) dose-dependent down-regulation in the serum levels of ALT and AST which demonstrates the hepatoprotective potential of Chloroform extract of *Argemone Mexicana*. The decrease in the levels of ALT and AST were more pronounced in animals administered with 100 and 200 mg/kg. It was also observed that the hepatoprotective activity of
Chloroform extract of *Argemone mexicana* particularly at a dose of 100 and 200 mg/kg was comparable to control group.

The effect of different doses (100 and 200 mg/kg) of Chloroform extract of *Argemone mexicana* was also determined on the levels of ALP and TP in the serum of all experimental animals and the results are shown in Table 1. Our results indicated that the exposure of animals to CCl₄ caused significant (P < 0.05) elevation in the ALP levels and a decrease in TP levels. The administration of Chloroform extract of *Argemone mexicana* caused a significant (P < 0.05) decrease in the levels of ALP and increased in the levels of TP at a dose of 100 and 200 mg/kg. A significant decrease in the levels of ALP and increase in the levels of TP showed promising hepatoprotective potential of Chloroform extract of *Argemone Mexicana*.

**Effect of Chloroform extract of Argemone mexicana on MDA levels in the serum**

In Chloroform extract of *Argemone mexicana* 100 and 200 mg/kg/p.o. (1.50±0.50; 1.30±0.50) treated group malondialdehyde levels (MDA) level decreased significantly (p < 0.05). In 10 mg/kg p.o. Silymarin (0.95±0.50) treated group MDA level decreased significantly (p < 0.05), respectively as compared with control group (2.50±0.80), as shown in Table 2.
Table 1: Effect of Chloroform extract of Argemone Mexican on biochemical evaluation in serum in CCl₄ induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose</th>
<th>ALT (%)</th>
<th>AST (%)</th>
<th>ALP (μL)</th>
<th>TP (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>0.5% CMC 1 ml/kg, p.o.</td>
<td>90.0 ± 9.74</td>
<td>90.0 ± 9.74</td>
<td>90.0 ± 10.30</td>
<td>90.0 ± 11.52</td>
</tr>
<tr>
<td>II</td>
<td>Control ( CCl₄)</td>
<td>2 mL/kg b.wt.</td>
<td>250.7 ± 6.01</td>
<td>268.05 ± 6.50</td>
<td>215.0 ± 8.00</td>
<td>63.3 ± 6.61</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin</td>
<td>10 mg/kg p.o.</td>
<td>141.0 ± 6.00***</td>
<td>133.0 ± 9.00***</td>
<td>145.00 ± 5.50</td>
<td>85.00 ± 5.50**</td>
</tr>
<tr>
<td>IV</td>
<td>Chloroform extract of Argemone mexicana</td>
<td>100 mg/kg p.o.</td>
<td>193.0 ± 5.50**</td>
<td>182.0 ± 7.00**</td>
<td>178.0 ± 6.50*</td>
<td>73.00 ± 6.50*</td>
</tr>
<tr>
<td>V</td>
<td>Chloroform extract of Argemone mexicana</td>
<td>200 mg/kg p.o.</td>
<td>175.0 ± 6.60**</td>
<td>170.0 ± 6.50**</td>
<td>165.00 ± 7.00*</td>
<td>69.00 ± 7.50*</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SEM of six observations. *** P<0.001 vs. control treatment (One-way ANOVA followed by Dunnett's test)
Table 2: Effect of Chloroform extract of *Argemone mexicana* on malondialdehyde levels (MDA) level in CCl₄ induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose</th>
<th>MDA μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>0.5% CMC 1 ml/kg, p.o.</td>
<td>0.60±0.50</td>
</tr>
<tr>
<td>II</td>
<td>Control (CCl₄)</td>
<td>2 mL/kg b.wt.</td>
<td>2.50±0.80</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin</td>
<td>10 mg/kg p.o.</td>
<td>0.95±0.50 **</td>
</tr>
<tr>
<td>IV</td>
<td>Chloroform extract of <em>Argemone mexicana</em></td>
<td>100 mg/kg p.o.</td>
<td>1.50±0.50 *</td>
</tr>
<tr>
<td>V</td>
<td>Chloroform extract of <em>Argemone mexicana</em></td>
<td>200 mg/kg p.o.</td>
<td>1.30±0.50 *</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett’s test).

**Phytochemical screening**

**Discussion**

Liver is one of the vital and visceral organs of the body, which is playing a very important role in maintaining body’s internal milieu and metabolic activity. In addition to its metabolic function it is able to store and release variety of endogenous substrates, vitamins, minerals, etc. It is also having a role in protecting the body from infectious microorganisms by detoxification and excretion of microbes and microbial products.¹⁵-¹⁶

CCl₄ is a hepatotoxic compound causing severe liver injury. It undergoes metabolism by the action of cytochrome p450 that is present in endoplasmic reticulum of liver cells and leads to the production of unstable and complex metabolites of CCl₄ which may cause hepatotoxicity. CCl₄ is activated in the presence of cytochrome p450 (CYP 2E1), and (CYP2B, CYP3A) both are marginally involved in the transformation of CCl₄ to its metabolites such as trichloromethyl (CCl₃-) free radicals that can also convert into trichloromethyl peroxy radical (CCl₃OO−) in the presence of oxygen. These metabolites of CCl₄ are very reactive. CCl₃ free radical covalently binds to the biomacromolecules and CCl₃O₂- involves in lipid peroxidation to dissolve the polyunsaturated fatty acid and change into small fragment called MDA or 4-hydroxynonenal. The reactivity of these free radicals alters the integrity or permeability of cell membrane due to oxidation of polyunsaturated fatty
acid in cellular membranes. This causes leakage of liver enzymes such as ALT, AST and ALP into the blood circulation.7

Therefore, in CCl₄-induced hepatotoxicity, the normal liver functions are affected which include substantial increase in the levels of liver enzymes such as ALT, AST, ALP, and TP. The levels of these liver enzymes and TP concentration are the main biochemical markers which indicate the status of liver function. Low levels of these biomarkers are normally present in the blood; however, in case of hepatotoxicity or injury to liver, levels of these biomarkers elevated in the blood. Therefore, the levels of ALT, AST, ALP and TP in the blood are directly related to the extent of the liver tissue damage.18 Our study results are accordance with study conducted by Pandey et al. which shown hepatoprotection by reducing the activities of SGOT, SGPT, and ALP, and bilirubin of CCl₄ intoxicated animals.19 Another study conducted by Rai et al., which is agreed with the present study results.20 However, our results indicated that treatment of CCl₄ intoxicated rats with chloroform extract of Argemone mexicana reversed the situation and resulted in a significant downregulation in the serum levels of these liver enzymes. These results clearly indicate the strong hepatoprotective activity of chloroform extract of Argemone Mexicana.

MDA is a direct biochemical marker of oxidative stress induced by chemicals, drugs, or external noxious injuries. The attack of free radicals on polyunsaturated membrane lipids cause the production of MDA which is measured as the product of free radical injury on membrane lipid.21 Our results indicated that the treatment of experimental animals with chloroform extract of Argemone mexicana cause significant decrease in the levels of MDA in the serum. These results indicated hepatoprotective efficacy of chloroform extract of Argemone Mexicana.

CCl₄ is one of the most commonly used toxins to induce hepatotoxic injury. Therefore, in this study, we ingested CCl₄ orally to Wistar albino rats for the purpose of inducing hepatotoxicity. The hepatoprotective efficacy of chloroform extract of Argemone mexicana was investigated by evaluating its potential to alleviate the elevation of liver enzymes (ALT, AST, and ALP) and enhance the antioxidant defense against oxidative stress by measuring MDA levels in the serum. We observed that chloroform extract of Argemone mexicana exhibit promising dose-dependent potential to alleviate the elevation of liver enzymes and main their homeostasis in the serum. We have also observed that the administration of chloroform extract of Argemone mexicana causes significant role in maintaining the optimistic antioxidant defense by reduced the level of MDA, which is a main indicator of hepatic tissue injury.

Conclusion

In conclusion, we demonstrated that daily dose of 100 or 200 mg/kg shows greater protective potential against CCl₄-induced hepatotoxicity and thus can be used as an
alternative natural hepatoprotective agent. The chloroform extract of *Argemone mexicana* exerts improvement in liver function by enhancing the antioxidant defence system capacity.

**References**


